CLIX. THE INFLUENCE OF SUCCINIC, FUMARIC, MALIC AND ACETIC ACIDS ON THE DEPOSITION OF LIVER-GLYCOGEN.

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(*Received July 6th, 1932.*)

The formation of succinic acid from pyruvic but not from acetic acid in muscle perfusion experiments led Toenniessen and Brinkmann [1930] to suggest this acid as an intermediate product in the oxidation of sugar *in vivo*. In the organism the part of succinic acid as an intermediate substance in the possible transformation of fat to sugar has been suggested by Clutterbuck and Raper [1925] and as a possible degradation product of the unsaturated acids by Smedley-MacLean and Pearce [1931]. Information as to the fate of succinic acid in the animal body is therefore of importance.

In the phloridzinised dog, feeding with succinic and malic acids resulted in the excretion of extra sugar [Ringer, Frankel and Jonas, 1913; Cremer, 1913] whereas feeding with malonic acid gave a negative result. Although Geelmuyden [1920] obtained extra sugar when acetic acid was administered to a phloridzinised dog, his results have not been confirmed by other workers [Deuel and Milhorat, 1928; Ringer and Lusk, 1910]. Thunberg's [1920] suggestion that succinic is formed from acetic acid in the body is therefore improbable. We investigated the effect of adding the ammonium salts of the four acids, succinic, fumaric, malic and acetic separately to a diet rich in fat and poor in carbohydrate which was fed to normal rats for 3 days following 24 hours' starvation. The glycogen and fat contents of the livers were then determined. In some experiments, a quantity of glucose of equal calorific value replaced the above acids.

The control diet and that to which sugar had been added were much better taken by the rats than the diets to which the salts of the acids had been added. It will be seen from Table I that in many cases in which the percentage of glycogen was increased the diet consumed was considerably less than in the controls. Rats about 6 weeks old were used, those for each experiment being from the same litter. The controls received a basal diet consisting of:

Bran (washed to remove most of the carbohydrate) ...	1·9 g.
Agar-agar	0·7
Bloater paste	0·5
Butter	3·0
Moisture	8·6
Total per diem ...	14·7

The experimental rats were given each day 2 g. of the ammonium salt of one of the acids to be tested in addition to the basal diet. The sugar controls received in addition to the basal diet 1·38 g. glucose, this being approximately equicalorific to 2 g. of ammonium succinate. After three days the rats were killed by a blow on the back of the head. The liver was rapidly removed, sliced, divided into two portions, one for estimating glycogen and one for fat: each portion was weighed and put into boiling 60 % KOH. The glycogen was precipitated, hydrolysed and estimated following the method of Evans, Tsai and Young [1931] the glucose being determined by the Shaffer-Hartmann method [1920–21]. The KOH solutions for fat estimations were acidified with hydrochloric acid, completely extracted with light petroleum and ether and the residues weighed. In order more accurately to determine the I.V. of the fats in Exp. 2, the acidified KOH solutions were extracted with carbon tetrachloride in the manner described by Hynd and Rotter [1930]. The extracts were made up to a definite volume and portions were titrated with $N/30$ alcoholic KOH, an equal volume of alcohol being added to the carbon tetrachloride solution before each titration. The fat content was calculated assuming a mean molecular weight for the fatty acids of 280.

The details of the experiments are shown in Table II and the collected results in Table I. The average glycogen percentages group themselves fairly sharply into three classes: (1) the percentages in the rats fed with the control diet and with the control diet *plus* acetate were respectively 1·08 and 1·26; (2) in those fed with the control diet to which succinate, malate or fumarate had been added, respectively 2·27, 2·18 and 2·18; (3) in those fed with the control diet and sugar the average percentage was 3·5. The *dl*-malate gave somewhat higher figures than the *l*-form. Clutterbuck [1927] had found that succinic acid incubated with minced liver yielded 75 % *l*-malic and 25 % fumaric acid. The fact that in our experiments *dl*-malic acid led to a higher percentage of glycogen in the liver than the *l*-form was therefore unexpected.

The average percentage of fat in the livers was markedly higher in the controls but no significant differences between the percentages in the other groups could be detected: the figures for the group which had received the additional sugar and for that which had received the acetate were similar.

Conclusion.

These results furnish evidence that in the normal rat the addition of succinic, malic or fumaric acid to a diet poor in carbohydrate leads to an increase of glycogen in the liver. After the addition of acetic acid no significant increase in glycogen could be detected.

The feeding experiments were carried out by Miss E. M. Hume and Miss Henderson Smith, whose co-operation and help we gratefully acknowledge.

We desire to acknowledge a grant from the Department of Scientific and Industrial Research which made it possible to carry out this work.

Table I.

No. of exp.	Diet (basal) (wt. in g. per day)			% glycogen in the liver						% fat in the liver					
	Carbo-hydrate	Protein	Fat (butter)	Control	Acetate	Fuma-rate	Malate	Suc-cinate	Sugar control	Control	Acetate	Fuma-rate	Malate	Suc-cinate	Sugar control
1	0·910	0·327	3	1·31 ♀	0·89 ♀	—	—	—	—	—	—	—	—	—	—
				1·30 ♀	1·54 ♀	—	—	—	—	—	—	—	—	—	—
					1·74 ♀	—	—	—	—	—	—	—	—	—	—
2	—	—	—	1·18 ♀	2·55 ♂	—	—	2·46 ♂	—	*5·27 ♀	4·47 ♂	—	—	4·07 ♂	—
				0·95 ♂	—	—	—	—	—	7·43 ♂	—	—	—	—	—
				0·22 ♀	—	—	—	—	—	11·05 ♀	—	—	—	—	—
3	—	—	—	—	0·74 ♀	—	*l-* 1·72 ♀	1·49 ♀	2·11 ♀	—	6·89 ♀	—	*l-* 5·16 ♀	4·95 ♀	4·15 ♀
					1·09 ♀	—	*l-* 2·31 ♂	1·45 ♂	0·65 ♀	—	5·89 ♀	—	*l-* 3·50 ♂	3·31 ♂	4·68 ♀
4	0·937	0·326	3	—	2·00 ♂	—	*l-* 1·05 ♀	0·10 ♀	1·63 ♀	—	4·81 ♂	—	*l-* 5·69 ♀	7·98 ♀	9·29 ♀
					0·56 ♀	—	*l-* 2·31 ♂	2·63 ♂	2·28 ♀	—	6·92 ♀	—	*l-* 4·16 ♂	5·17 ♂	7·11 ♀
5	0·950	0·347	3	1·88 ♀	—	0·58 ♀	*dl-* 3·42 ♂	4·67 ♂	4·88 ♀	10·16 ♀	—	4·60 ♀	*dl-* 3·65 ♂	3·07 ♂	6·69 ♀
						2·13 ♂	*l-* 1·41 ♀	—	4·20 ♀	—	—	3·49 ♂	*l-* 4·51 ♀	—	4·34 ♂
6	0·948	0·350	3	1·06 ♀	—	2·89 ♂	*dl-* 2·16 ♂	1·35 ♀	2·98 ♀	7·76 ♀	—	3·12 ♂	*dl-* 4·35 ♂	4·64 ♀	5·42 ♀
						2·23 ♀	*l-* 2·38 ♂	—	5·04 ♀	—	—	5·88 ♀	*l-* 3·79 ♂	—	5·32 ♀
7†	0·805	0·339	3	0·76 ♀	0·22 ♀	3·05 ♀	*dl-* 3·44 ♂	2·38 ♀	3·44 ♀	7·24 ♀	5·27 ♀	4·43 ♀	*dl-* 4·81 ♂	1·87 ♀	3·80 ♀
						2·20 ♂	*l-* 1·63 ♀	4·06 ♀	4·31 ♀	—	—	2·70 ♂	*l-* 4·99 ♀	4·61 ♀	5·10 ♀
Mean	0·910	0·338	3	1·08	1·26	2·18	2·18	2·27	3·50	8·15	5·71	4·04	4·46	4·41	5·59

* By CCl_4 extraction and titration.

† Albinos; all the other litters were black and white.

Table II.

(Protocols.)

No. of exp.	Diet	Sex	Original weight g.	% loss of weight	% food eaten	% glycogen	% fat
1	Control	♀	90	3	100	1·31	
	Control	♀	78	2	100	1·30	—
	Acetate	♀	78	6	84	0·89	—
	Acetate	♀	94	7	72	1·54	—
	Acetate	♀	95	6	66	1·74	—
2	Control	♀	120	4	100	1·18	5·27*
	Control	♂	139	6	100	0·95	7·43
	Control	♀	105	3	100	0·22	11·05
	Acetate	♂	157	6	100	2·55	4·47
	Succinate	♂	149	9	94	2·46	4·07
3	Acetate	♀	102	9	98	0·74	6·89
	Acetate	♀	103	4	92	1·09	5·89
	Succinate	♀	105	10	82	1·49	4·95
	Succinate	♂	104	16	88	1·45	3·31
	l-Malate	♀	120	5	99	1·72	5·16
	l-Malate	♂	110	13	82	2·31	3·50
	Sugar control	♀	97	6	63 (rat pneumonia)	2·11	4·15
	Sugar control	♀	98	5	98	0·65	4·68
4	Acetate	♂	118	8	86	2·00	4·81
	Acetate	♀	119	7	90	0·56	6·92
	Succinate	♀	130	13	60	0·10	7·98
	Succinate	♂	130	12	44	2·63	5·17
	l-Malate	♀	125	7	90	1·05	5·69
	l-Malate	♂	138	9	78	2·31	4·16
	Sugar control	♀	120	4	100	1·63	9·29
	Sugar control	♀	121	0	100	2·28	7·11
5	Control	♀	70	2	98	1·88	10·16
	Succinate	♂	83	9	87	4·67	3·07
	dl-Malate	♂	80	9	66	3·42	3·65
	l-Malate	♀	75	9	78	1·41	4·51
	Fumarate	♀	82	12	57	0·58	4·60
	Fumarate	♂	76	9	69	2·13	3·49
	Sugar control	♀	72	1	99	4·88	6·69
	Sugar control	♂	70	0	91	4·20	4·34
6	Control	♀	105	7	100	1·06	7·76
	Succinate	♀	120	9	68	1·35	4·64
	dl-Malate	♂	140	12	49	2·16	4·35
	l-Malate	♂	138	11	79	2·38	3·79
	Fumarate	♂	115	10	49	2·89	3·12
	Fumarate	♀	117	10	79	2·23	5·88
	Sugar control	♀	115	2	100	2·98	5·42
	Sugar control	♀	110	0	100	5·04	5·32
7†	Control	♀	108	2	100	0·76	7·24
	Acetate	♀	112	4	84	0·22	5·27
	Succinate	♀	101	4	94	2·38	1·87
	Succinate	♀	108	5	72	4·06	4·61
	dl-Malate	♂	125	6	84	3·44	4·81
	l-Malate	♀	123	5	92	1·63	4·99
	Fumarate	♀	113	4	80	3·05	4·43
	Fumarate	♂	120	6	68	2·20	2·70
	Sugar control	♀	108	Gain of 2	100	3·44	3·80
	Sugar control	♀	103	Gain of 2	92	4·31	5·10

* In this group fats were estimated by extraction with carbon tetrachloride and titration with sodium hydroxide.

† Albinos.

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